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PROTOCOL FOR DERMAL TOXICITY TESTING FOR MEDICAL DEVICES IN CONTACT WITH SKIN

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This guidance document may contain references to addresses and telephone numbers that are now obsolete. The following contact information is to be used instead:

- **While this guidance document represents a final document, comments and suggestions may be submitted at any time for Agency consideration to the Orthopedic Devices Branch, 9200 Corporate Blvd., HFZ-410, Rockville, MD 20850.**
- **For questions regarding the use or interpretation of this guidance, contact the Orthopedic Devices Branch at 301-594-2036.**
- **To contact the Division of Small Manufacturers Assistance (DSMA), call 800-638-2041 or 301-443-6597; fax 301-443-8818; email dsmo@cdrh.fda.gov; or write to DSMA (HFZ-200), Food and Drug Administration, 1350 Piccard Drive, Rockville, Maryland 20850-4307. FACTS-ON-DEMAND (800-899-0381 or 301-827-0111) and the World Wide Web (CDRH home page: http://www.fda.gov/cdrh/index.html) also provide easy access to the latest information and operating policies and procedures.**

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Assessment of the potential dermal toxicity on a substance may include primary irritation, corrosion, cumulative irritation, sensitization, as well as systemic toxicity tests. Initial screening of individual chemicals or mixtures not previously used in topically applied products should be conducted in animals to avoid needless exposure of humans to substances obviously unsuitable for dermal use; humans should be used in final product. testing to determine its potential adverse effects particularly on a high risk group such as Caucasian skin. In order to minimize the variations inherent in tests using subjective rating systems, all dermal toxicity testing should be done by experienced Personnel according to a predetermined protocol.

This protocol has been developed specifically for testing of topically applied electrodes, electrode adhesives, pastes, gels, and creams. The product to be tested (liquid, solid, or semisolid) should represent a homogeneous aliquot of the substance under investigation. This is especially important when testing formulated products containing several ingredients, such as emulsions or suspensions which can separate into different substances during standing, shipping, or temperature change.

PRELIMINARY INFORMATION

I. Chemical Composition of any parts of a device, such as the electrode and/or electrode adhesives, paste, gel or cream in contact with skin including the concentrations of the different ingredients and impurities present, and the previous use of these components in other dermal products should be documented. Products for dermal use should maintain the physiological range of pH 5-8, Healthy skin can adjust to a wide range of hydrogen ion concentrations through its natural buffering capacity. (9) Adverse effects resulting from buffering problems should be manifested as irritation or corrosion in dermal toxicity studies.

II. A bibliography of medical literature and other information pertaining to toxicity studies demonstrating the safety or any adverse effects of chemicals used in the device should be prepared, Copies of articles of major interest should be submitted to the Bureau along with test results.

ANIMAL TESTING

I. **Systemic Toxicity - Acute Dermal Toxicity**. Chemicals which pass through the skin may enter the circulation and cause general toxic effects. If the potential toxicity of a chemical is unknown or a chemical is known to be toxic by other routes of administration, acute dermal toxicity studies should be conducted to determine the median lethal dose (LD50) resulting .from dermal exposure of animals to a chemical or mixture. (1) This test would be appropriate for substances used in devices which are in continuous contact with the skin for extended periods of time; for devices in short term use, i.e., less than 6 hours in contact with a small portion of the body surface, testing would be probably not appropriate.

II. **Dermal Toxicity Testing** - The components should he tested in the formulation of intended use. New formulations consisting of previously used components should also be

tested to determine whether 'any adverse effects result from a combination of components not exhibiting adverse Effects when used separately or in other products. Concentrations of individual components of electrode gels or adhesives can be increased to allow for a safety factor in estimation of the potential for adverse effects of a substance slated for multiple applications to patients. In selecting animal test conditions, the conditions of the skin of potential patients for the device should be considered since injured or diseased skin exhibits greater absorption of materials and may show greater reactivity. Abraded skin caw--be used in animal studies to increase absorption of the test substance and the sensitivity of the test; abrasion techniques involve incising the stratum corneum but bleeding should not occur. Since abrasion techniques are difficult to interpret and standardize they are not usually recommended. (7)

A. Primary Irritation and Corrosion - Primary irritation of the skin can be defined as a local inflammatory reaction which does not produce tissue destruction or irreversible change at the site of contact; the macroscopic manifestations of irritation are edema and erythema. (1) The albino rabbit is the most commonly used animal for this test. Tests should be conducted and scored as described in the Federal Register. (3) Because of inter-laboratory variation in absolute test scores, the score should be evaluated in comparison with a known irritant and non-irritant with similar chemical and physical properties to the test material.

Since isolated cases of corrosion from devices have been reported, this discussion has been included. Corrosion is defined as visible destruction or irreversible alteration in tissue at the site of contact manifested by ulceration, necrosis, and eventual formation of scar tissue with exposures for 4 hours or less. (6) Mixtures designed for topical application will probably not be corrosive. The rabbit is the animal used to determine if a substance is corrosive. Exposure of the animal to a potentially corrosive substance should relate to the intended exposure of the patient to the product. For corrosion it may be necessary to evaluate the effects up to seven days after patch application. Corrosive substances should not be used in devices designed for contact with human skin. Testing of potentially corrosive substances in humans should not be undertaken because of the inherent danger involved. (1)

B. Cumulative Irritation - If the intended final use of a product involves recurrent exposures to a material, cumulative irritation studies in animals may be a more realistic model for study of potential skin irritation of substances not exhibiting primary irritation. Cumulative irritation is defined as primary irritation arising from recurrent exposures to substances which do not cause acute primary irritation on a single exposure. (6) Daily applications of 5-7, 14 or 21 days have been used in cumulative irritation tests in animals. (6) For the greatest margin of safety 21 days should be used.

C. Sensitization - Several animal models have been developed for identification of contact allergens in animals including the Draize test, Freund's Complete Adjuvant Technique, the Buhler Test and the open Epicutaneous Test. (6) The naive guinea pig is the most commonly used animal for sensitization tests since its response level is higher than other animals and similar to humans. (7) In the

traditional Draize test in guinea pigs, potent sensitizers are identified but moderate sensitizers in humans may give false negative reactions in guinea pigs. Use of adjuvant in the guinea pig maximization test or the split adjuvant technique gives a lower probability of missing a sensitizer. (6) If a substance is a sensitizer in the traditional Draize test it is not necessary to perform the maximization test. If a substance is negative in the traditional Draize test, then a guinea pig maximization test should be run since use of adjuvant makes the test more sensitive.

In evaluating the sensitization reactions, comparison of exposure of the challenge group with an exposure to a naive control group of animals of the same age as the test group should be made in order to distinguish between irritation and sensitization. Usually twenty naive guinea pigs each are used in the experimental and control groups. If the formulation .intended for the final product is used in testing the concentration of a substance suspected of causing sensitization can be increased ten-fold in the formulation during induction as a safety factor in conducting sensitization studies; the original formulation should be used during challenge. The amount of the test substance applied to the guinea pig during challenge should not exceed the highest amount which is non-irritating in naive guinea pigs in a 24 hour irritation study. Also, if a particular chemical formulation is found to cause sensitization upon challenge then a second challenge 1-2 weeks after primary challenge can be conducted on individual components of the mixture to identify the sensitizing substance.

Substances exhibiting strong sensitization in guinea pigs are likely to cause a substantial number of sensitization reactions to man. The tendency for weak sensitizers of guinea pigs to produce substantial numbers of sensitization reactions in man can not be predicted. Substances causing weak sensitization in guinea pigs should be tested in humans before being used in marketed products.

HUMAN TESTING

In all investigations using human subjects informed consent is required. Each subject should receive printed information about the test to be performed and a medical history of each subject should include information on any cases of dermatitis, sensitivities to specific substances, and present medications such as immuno-suppressive drug' which would contraindicate subjects for the study.

I. **Irritation -** In most cases, screening substances in rabbit irritation studies will avoid problems with humans. After a potentially suitable formulation has been identified as a result of irritation tests in animals, studies on human volunteers should proceed.

A greater number of human than animal subjects is necessary because of the greater variability in human skin. Acute primary irritation studies are usually conducted using 10-60 human volunteers. Some investigators favor testing with a preselected group of highly reacting (Caucasian) individuals since the effect of a formulation on the population at risk may be the determining factor in deciding whether a product is suitable for marketing. (5) Alternately, scarification can be used to assess the potential irritation of a

substance on sensitive or diseased skin but this technique may result in permanent effects on skin pigmentation and is not recommended. Sodium lauryl sulfate is also used to increase skin reactivity, but is not recommended because of the potential for sensitizing subjects to substances unnecessarily. Acute irritation studies in humans usually involve application of substances under patches on the intrascapular area of the back of the dorsal surface of the upper arms for four hours. Using 8-10 patches per subject allows for use of comparison standards in one or more positions. The order of patches on subjects should be varied in order to eliminate variations caused by pressure of clothing or furniture on the site.

Evaluation of the responses is done 30 minutes to 1 hour, and 24 hours after removal of the patch. Another reading is made 3 to 4 days after application to check for delayed or persistent reactions. Several scales for scoring human skin responses have been developed. (8,7) Scores are compared with those produced by substances of known irritancy.

Cumulative irritation studies, usually done on 10 to 50 subjects, involve multiple exposures of 15-24 hours over a 21 day period. Evaluation is similar to that for primary irritation.

II. **Sensitization**

For sensitization studies in humans more subjects are required than in animal studies because of the greater variation in immune responses and the necessity of using lower concentrations of materials in human exposure. Many predictive test procedures have been developed with different variations in skin preparation, concentrations to be used, and the number of subjects required. (6) Usually the sensitization of a substance is evaluated in a pilot group of 20-25 subjects first and then expanded to a larger group of up to 200 subjects to avoid unnecessary exposure if the substance is a strong sensitizer in the pilot group. The sample size of test subjects must be large enough so that the results are valid for the population for which the preparation is intended. It may be of interest to use a particular component at a level ten times the concentration in the finished product for induction; for challenge a non-irritating dose should be used. A dose-response relationship should be established in order to predict the likelihood of occurrence of sensitization with a particular product, the test conditions should reflect the actual use of the product, i.e., substances should be tested in the presence of all components in contact with the skin and under conditions of use such as in TENS with current flowing through electrodes at the maximum level of intended use. In general sensitization procedures require 24 to 48 hour multiple applications of occlusive patches for the induction phase; 9 to 15 applications are made over a 3 week period. Induction is followed by a 10-14 day rest phase to allow for development of latent sensitization. Subjects are then challenged by application of the test material at a different site for 48 hours. Responses are evaluated 1, 24, 48 and 96 hours after application of the patches. If results are positive, a second challenge 2 weeks after the original challenge may be conducted. At this time a particular component suspected as the sensitizer may be tested alone at different concentrations to confirm identification of the sensitizing component of the product.

Evaluation must distinguish between primary irritation responses which may disappear within a couple of days and sensitization responses which may develop slower, persist longer and are characterized by induration, popules, or vesiculation. Further identification of sensitization reactions involves microscopic examination of skin biopsies. Some factors which may be encountered in human studies include reactions early in the induction phase which may be indicative of pre-existing sensitization to the test substance, delayed responses at 192 hours instead of 48 or 96 hours. Follow-up of subjects not completing the study may yield valuable information on adverse effects of a preparation.

REFERENCES

1. Principles and procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences Publication 11 38, 1 964.

2. Hazardous Substances: Proposed Revision of Test for Primary Skin Irritants, Federal Register, 37, No. 244, 27635-27636 (1972).

3. 38FR27012, September 27, 19 73 and 38FR30105, November 1, 1973.

4. Weil, C.S. and R.A. Scola, "Study of Intra- and Interlaboratory Variability in the Results of Rabbit Eye and Skin Irritation Tests, Toxicol. Appl. Pharmocal., 19, 276-360 (1970).

5. Loomis, Ted, Proceedings of the Conference on Cutaneous Toxicity, May, 1979, Washington, D. C., (In Press).

6. “Dermatotoxicology and Pharmocology," Advances in Modern Toxicology, Vol . 4, Edited by Francis A. Marzulli and Howard I. Maibach, John Wiley and Sons, New York, 1973.

7. Principles and Procedures for Evaluation of the Toxicity of Household Substances, National Academy of Sciences, Washington, D. C., 1977.

8. Marzulli, F.N. and H.I. Maibach, "The Rabbit as a Model for Evaluating Skin Irritants: A Comparison of Results Obtained on Animals and Man Using Repeated Skin Exposures," Food, Cosmetic Toxicology, 13, 533-540, (1979).

9. “Physiology and Biochemistry of the Skin," Stephen Rothman ED., University of Chicago Press, 227-232, (1954).